THE GENUS ONCHOCOTYLE, WITH A DESCRIPTION OF ONCHOCOTYLE WARDI, N. SP.

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY David Causey.

ENTITLED The Genus Onchocotyle, with a Description of Onchocotyle Wardi, N. Sp.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Arts.

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*Required for doctor's degree but not for master's

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I. Foreword

This research was undertaken in the Laboratory of Parasitology at the University of Illinois, under the direction of Doctor Henry B. Ward, to whom the writer wishes to express his sincere appreciation of the interest shown in the work, and for the furnishing of the material which made it possible. The writer also acknowledges many helpful suggestions from Mr. D. C. Hetherington.

The specimens used in this research were obtained from the gills of the Sleeper Shark, Somniosus microcephalus Block, at Excursion Inlet, Alaska, on July 26, 1909. The Sleeper Shark is also the host of Onchocotyle borealis, the species most nearly resembling the new species. It is known in European waters as the Greenland Shark. Goode, speaking of this shark, says:

"This species, also called by our fishermen the "Gurry" or "Ground" Shark, is a native of the Arctic Seas, but on our coast ranges south to Cape Cod, and in the Eastern Atlantic at least to England, while in the Pacific it has been observed from Puget Sound northward."

II. Technique

The parasites as obtained for study were in 85% alcohol, having been killed with corrosive acetic fixing fluid. The excess of corrosive sublimate was removed with iodine in alcoholic solution in the usual manner. For toto preparations the worms were over-stained in paracarmine or Delafield's haematoxylin, and then destained in acid alcohol. After neutralization with alkaline alcohol (NH_4OH), the specimens were cleared in synthetic oil of wintergreen and mounted in balsam. Xylol, cedar oil, and clove oil were tried in addition to the oil of wintergreen, but the latter gave the most satisfactory results. The worms stain intensely, and best differentiation was obtained after the mounts were finished by exposing the slides to sunlight. This brought about a gradual destaining that brought out the reproductive system in a satisfactory manner. For sections, Delafield's haematoxylin with erythrosin as a counter-stain gave the desired contrast. The digestive system takes the stain very poorly, the dark granules are quite unaffected, which is also the case of the vitellaria. In both cases, the natural coloration is quite sufficient. The mesenchyma and reproductive systems take the stain readily.

The toto preparations, because of their great thickness, could not be studied to any extent under the compound microscope. Instead a binocular dissecting microscope was used and served the purpose very well.

III. The Genus Onchocotyle Diesing 1850

The genus *Onchocotyle* was first established by Diesing in 1850, based on the trematode discovered by Kuhn in 1829 and named by him *Polystoma appendiculatum*. This is now *Onchocotyle appendiculatum*. The genus was originally described by Diesing as follows:

"Corpus lineari - lanceolatum depressum, utrinque angustatum. Caput corpore continum. Os subterminale. Acetabula sex disco elliptico extremitati caudali supra adnato, biseriatim immersa, hemisphaerica, margine uncino simplici inferna adnato apice libero armata. Aperturae genitales . . . Porus excretorius in apice caudali. Piscium marinorum ectoparasita."

Goto's definition in 1894 is as follows:

"Body elongated, very thick, narrow towards both ends; anterior end blunt, and with a sub-ellipsoidal sucker around the mouth cavity; with three pairs of circular or elliptical suckers at the posterior end, each with a semi-circular chitinous supporting piece with one of the ends provided with a claw. With a sub-cylindrical caudal appendage, which bears at its extremity a pair of small suckers destitute of any chitinous framework; often with a pair of hooks between these suckers. With a paired vaginal opening on the ventral side of the body. Porus genitalis ventral and median."

The following species have been described:

Onchocotyle abbreviata Olsson 1876.
" appendiculata (Kuhn, 1830) Diesing 1850.
" borealis van Beneden 1853.
" canis (Cerfontaine, 1899) Pratt 1900.
" emarginata Olsson 1876.
" scymni ainosi Diesing 1858.
" spinacis Goto 1894.
" canicula (Cerfontaine, 1899).
" vulgaris (Cerfontaine, 1899).
" griesa (Cerfontaine, 1899).
" batis (Cerfontaine, 1899).
" prenanti St. Remy 1890.
" alba (Cerfontaine, 1899).

Of these, O. emarginata Olsson 1876 has been shown to be synonymous with O. appendiculata. O. scymni ainosi was listed by Diesing in 1858, with the remark that there was no description of this species. Obviously a named species without a description cannot be given serious consideration.

IV. The Systematic Position of the Genus Onchocotyle

The systematic position of the genus Onchocotyle is quite typical for so many genra of parasites first described early in the last century and hastily assigned systematic positions without careful study. Scarcely an investigator has worked with this genus without changing the nomenclature and systematic position.

The first species of this genus was described by Kuhn in 1829 as Polystoma appendiculatum. Diesing established the genus Onchocotyle in 1850, and Polystoma appendiculatum became Onchocotyle appendiculatum. Van Beneden in 1853 described a new species of the Onchocotyle which he had found on the gills of Scimnus glacialis and named Onchocotyle borealis, a genus of the family Polystomidae. The systematic position was clearly shown by him in 1858 when he proposed a classification of trematodes which gained general acceptance and in its main features is the one recognized today. Van Beneden divided the order of trematodes into two sub-orders: the Monogenea, which develop directly without metamorphosis, and are largely ecto-parasitic; and the Digenea, which undergo metamorphosis, and are mainly endoparasitic. He divided the sub-order Monogenea into two families: the Tristomidae being forms with a single posterior sucker; and the Polystomidae, forms with two or more posterior suckers. The following is adapted from his paper of 1858:

Order	Sub-order	Family	Genra
Trematoda	I. Monogenea	I. Tristomidae	I. Udonella
			II. Epibdella
			III. Tristoma
		II. Polystomidae	I. Diplozoon
			II. Octobothrium
			III. Axine
			IV. Onchocotyle
			V. Polystomum
			VI. Calceostoma
			VII. Gyrodactylus
	II. Digenea		

In 1863 Van Beneden and Hesse revised and expanded the earlier classification as follows:

Order	Sub-order	Family
Trematodes	Monogenea	Tristomidae
		Polystomidae
		Octocotylidae
		Uronellidae
		Gyrodactylidae

Onchocotyle was retained as a genus under the family Polystomidae as in the original classification.

Monticelli proposed in 1892 a new classification of trematodes which was adopted by most of his fellow workers with some modification, and which was in vogue for a number of years. He discarded the older division into the Monogenea and Digenea, and instead made three great groups or sub-orders.

Order	Sub-order
I. Trematoda	I. Heterocotylea
	II. Aspidocotylea
	III. Malacotylea

The Heterocotylea were practically the same as the Monogenea, while under the Aspidocotylea and Malacotylea were grouped the members of the Digenea. The Heterocotylea were subdivided into a large number of families, among which were the Polystomidae and the Octocotylidae. The Onchocotyle were taken from the Polystomidae, and placed among the Octocotylidae, the sub-family Onchocotylinae being created. The Onchocotylinae included three genera: Squalonchocotyle, Onchocotyle, and Rajonchocotyle.

It is interesting to note that in the same year, 1892, Saint-Remy's classification not only was based on that of van Beneden, but made the family Polystomidae include both the Octocotylinae and the Polystominae. The Onchocotyle were placed as a genus in the sub-family Polystominae. His classification was:

Order	Sub-order	Family	Sub-family
I. Trematoda	I. Monogenea	I. Temnocephalidae	
		II. Tristomidae	
		III. Polystomidae	
			I. Octocotylinae
			II. Polystominae

Braun (1893) agreed with Saint-Remy as to the classification of the *Onchocotyle*, the general classification being identical with that of van Beneden, i.e., the sub-orders *Monogenea* and *Digenea* were retained.

Cerfontaine in 1899 placed in the *Octocotylidae* those forms having eight posterior suckers. The organs of the appendix having been shown to be suckers, he accordingly placed the *Onchocotyle* in this family, and created three genera: the *Squalonchocotyle*, the *Acanthocotyle*, and the *Rajonchocotyle*.

Monticelli's classification was adopted by Pratt in his key to the trematodes which appeared in 1900, and the *Onchocotyle* were again placed as a genus of the family Polystomidae:

Order	Sub-order	Family	Sub-family	Genus
I.Trematoda	I.Hetercotylea	I.Polystomidae	I.Polystomae	I.Onchocotyle

In the latest work dealing with the classification of trematodes, that of Ward in 1918, the original grouping of van Beneden into *Monogenea* and *Digenea* is used. His key shows the following classification:

Class - Trematoda.

Sub-class - Monogenea.

Order - Polyopisthocotylea (Odhner)

Families:

I. Octocotylidae

II. Microcotylidae

III. Polystomidae

He draws the following distinctions:

"With two oral suckers and with genital hooks,

Family Octocotylidae.

Family Microcotylidae.

"Anterior end pointed, without suckers or special organs,

Family Polystomidae."

This very clearly places the _Onchocotyle_ in the _Polystomidae_ and not in the _Octocotylidae_. Pratt in his key in 1900 also placed the _Onchocotyle_ under the _Polystomidae_ in a similar fashion.

The following, then, is the systematic position given to the Onchocotyle:

Class - Trematoda

Sub-class - Monogenea

Order - Polyopisthocotylea Odhner.

Family - Polystomidae van Beneden.

Genus - Onchocotyle.

V. Comparison of Onchocotyle Wardi, n. sp. with the Other Species of the Genus

In considering the various species of the genus *Onchocotyle* in order to test the position of the form studied, certain known species may be set aside at the beginning as obviously so different as to require little or no consideration. *O. batis*, *O. prenanti*, and *O. alba*, in which the digestive tract ramifies within the fixation disk, and *O. canicula*, and *O. appendiculata*, in which genital hooks are present, are evidently very different.

Goto emphasizes the importance of the hooks in classification and thinks that they alone are sufficient for identification. In separating and distinguishing the species of Onchocotyle I have relied upon the hooks to a great extent. Measurements were obtained by stepping off with a pair of dividers the distance along the median line from end to end and correcting for the magnification. Cerfontaine's figures were used for this with the exception of *O. wardi*, for which my own figure (Fig. 9), was used. The measurements of the eggs were obtained in a similar fashion, and in each case the greatest length and breadth is given. I am unable to find a record of the size of the egg of *O. appendiculata*. Van Beneden figures one but does not state the magnification. The egg is unknown in *O. spinacis*.

The preparation of the hooks for examination may introduce an error. If the hooks are isolated by treating the fixation

disk with lactic acid for a number of hours and then dissecting, the terminal elevations will have disappeared. If, however, the fixation disk is prepared in the usual way for toto mounts in balsam or in glycerine gelatine medium, the presence or absence of terminal elevations may be determined.

The dimensions of the hooks and eggs, and of the length of the body of the various species are:

Species	Length of Hooks	Length of Eggs	Width of Eggs	Length of Body
O. vulgaris	1428 μ	270 μ	110 μ	12 mm.
O. canis	700 μ	155 μ	60 μ	7 - 8 mm.
O. abbreviata	900 μ	150 μ	60 μ	7 - 8 mm.
O. spinacis	942 μ	Egg unknown		8 - 9 mm.
O. canicula	528 μ	220 μ	130 μ	
O. appendiculata	471 μ	No data obtainable		6 - 7 mm.
O. borealis	1685 μ	330 μ	120 μ	20 mm.
O. griesa	1514 μ	255 μ	67 μ	15 mm.
O. alba	1485 μ	225 μ	120 μ	8 - 9 mm.
O. prenanti	785 μ	260 μ	120 μ	8 - 9 mm.
O. batis	1800 μ	210 μ	105 μ	12 -15 mm.
O. wardi	1940 μ	296 μ	97 μ	17 -28 mm.

O. wardi has large hooks in the fixation disk which show a variable number of terminal elevations. Rarely a hook shows only three elevations, usually it has four or five. This variation may be found among the hooks of the same disk, where

the majority show four or five. The mature egg, as found in the uterus on its way to the genital atrium never shows more than one polar filament. This filament is never greater than the length of the body-proper, and usually is less, averaging in length about half of that of the body-proper. At the other pole is a knob-like body which varies in size (Fig. 30).

Of the species not already eliminated because of their more obvious differences, the following have specific characteristics that may be pointed out. O. canis has no terminal elevations on the hooks; O. vulgaris and O. abbreviata have two terminal elevations per hook, and two polar filaments on each egg; O. spinacis and O. griesa have three terminal elevations per hook, and the egg of the latter has two polar filaments (the egg of O. spinacis is unknown). O. borealis has four terminal elevations per hook and an egg with two polar filaments.

An inspection of the table shows that O. wardi stands apart from the great majority of the Onchocotyle because of its greater size, larger hooks and eggs. That species that approaches it most nearly is O. borealis, which is of approximately the same length, has a hook nearly as large, and an egg of greater size. A comparison between the two is necessary to bring out the specific differences.

O. borealis has not been carefully worked out, so that comparisons of the various systems is very difficult. Van Beneden's original description is the best, Cerfontaine gives nothing besides a careful description and illustration of the hooks and egg. The

general size of the body in the two species is the same. In O. borealis the anterior sucker is a wide, shallow and thin walled cup with a relatively large mouth in the centre. In O. wardi the anterior sucker is smaller in proportion to the size of the body, is thick walled and deep, and has a small mouth in the centre. Van Beneden does not mention a genital atrium and his figures do not indicate that he found one. In O. wardi there is a genital atrium (Fig. 5).

In the fixation disk certain differences are evident. In O. borealis the suckers of the fixation disk are very distinct, with the lower sides of each tapering slightly inward toward the base. In O. wardi this is not the case (Fig. 7), instead the sides of the suckers continue into the disk proper so as to show no distinct line of demarcation between the disk and the suckers. The difference in the suckers in each species is more striking. In O. borealis each sucker has a mouth that is distinctly divided into four large lobes or lips. In O. wardi the suckers are smaller and have smooth mouths without a trace of lobes.

As indicated in the table, the hooks of O. wardi are larger than those of O. borealis, while the eggs are considerably smaller. There is a further difference in that O. borealis has two polar filaments per egg, while O. wardi has only one. The variations in the number of terminal elevations has already been indicated.

Van Beneden did not have a clear conception of the relations the parts of the reproductive system have to each other in

this genus, and his figures show he misinterpreted various ducts. The genital-intestinal canal and the posterior end of the uterus are not only figured as vitellarian ducts, but are made to lead to the vitellaria. The ootype is figured as a "vitellosac." With these obvious errors corrected, one important difference may be pointed out. In O. borealis the lateral yolk-ducts unite and the common yolk-duct empties directly into the ootype at its anterior end. At the other end of the ootype, the genital-intestinal canal, the oviduct and the uterus arise. In O. wardi this is not the case. In O. wardi the common yolk-duct formed from the union of the lateral yolk-ducts, together with the genito-intestinal canal and the oviduct, all unite and a short duct leads to the ootype, joining it at its posterior end. The uterus arises from the anterior end. These differences are shown in the text figures 1 and 2.

The differences between O. borealis and O. wardi may be summed up briefly as:

1. A difference in the anterior suckers.

2. A genital atrium is present in O. wardi and not indicated in O. borealis.

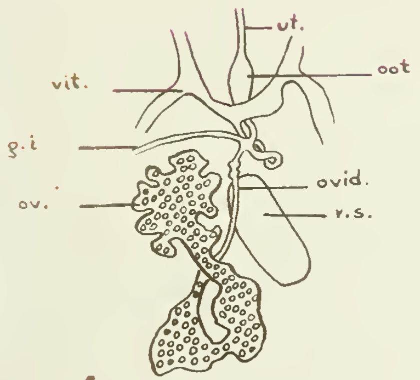
3. The eggs of O. borealis are larger and have two polar filaments, while the eggs of O. wardi have one polar filament each.

4. The hooks of O. wardi are larger than those of O. borealis.

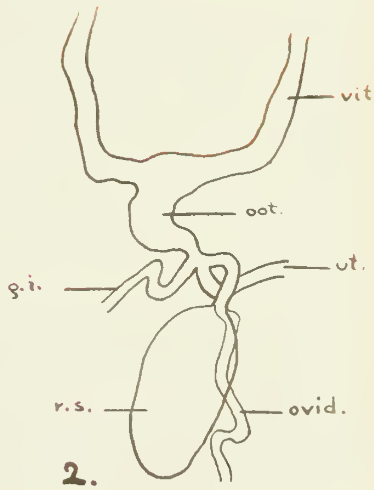
5. Each sucker of the appendix of O. borealis has a mouth with four lobes, while in O. wardi the mouth is not lobed.

6. There is a difference in the connection of the uterus, yolk-duct, oviduct and genital-intestinal canal with the ootype.

Text figures .



1.



2.

Figure 1. Onchocotyle wardi X20

Figure 2 Onchocotyle borealis

g.i genito-intestinal canal

oot. ootype
ov. ovary
ovid . oviduct
r.s. recapaculum seminale
ut. uterus

VI. Key to the Species of the Genus Onchocotyle

1 (17) Digestive tract does not ramify within the fixation disk 2

2 (5) Genital hooks present 3

3 (4) Large hooks of fixation disk with four terminal elevations, free end of hook tapers slightly. Genital hooks roughly T-shaped. Egg 220 µ in length, 130 µ in width, one polar filament. Large hooks 528 µ in length.

<u>O. canicula</u> (Cerfontaine, 1899).

4 (3) Large hooks of fixation disk with more than four terminal elevations, free end of hook slightly swollen. Genital hooks roughly L-shaped. Egg with two polar filaments. Large hooks 471 µ in length; body length, 6 - 7 mm.

<u>O. appendiculata</u> (Kuhn, 1830) Diesing 1850.

5 (2) Genital hooks absent 6

6 (7) Large hooks of fixation disk without terminal elevations, egg 155 µ in length, 60 µ in width, with two long polar filaments. Large hooks 700 µ in length; body length, 7 - 8 mm.

<u>O. canis</u> (Cerfontaine, 1899) Pratt 1900

7 (6) Large hooks of fixation disk with terminal elevations 8

8 (11,14) Each hook with two terminal elevations 9

9 (10) Egg with short equal polar filaments. Egg 270 µ in length, 110 µ in width. Large hooks 1428 µ in length; body length, 12 mm.

<u>O. vulgaris</u> (Cerfontaine, 1899)

10 (9) Egg with very long polar filaments. Egg 150 µ in length, 60 µ in width. Large hooks 900 µ in length; body length 7 - 8 mm.

<u>O. abbreviata</u> Olsson 1876

11 (8,14) Each hook with three terminal elevations 12

12 (13) Hooks on appendix Y-shaped, with the three prongs approximately equal, the two prongs embedded in mesenchyma diverging widely. Egg unknown. Large hooks 942 µ in length; body length, 8 - 9 mm. <u>O. spinacis</u> Goto 1894

13 (12) Hooks on appendix not Y-shaped, two embedded prongs very short. Egg 255 µ in length, 67 µ in width; with two short equal polar filaments. Large hooks 1514 µ in length; body length 15 mm.

<u>O. griesa</u> (Cerfontaine, 1899)

14 (8,11) Each hook with four or more terminal elevations . 15

15 (16) Suckers on appendix each with a mouth divided into four lobes. Terminal elevations four. Egg 330 µ in length, 120 µ in width; with two equal polar filaments. Large hook 1685 µ in length; body length, 20 mm. <u>O. borealis</u> van Beneden 1853

16 (15) Suckers on appendix with smooth circular mouths; terminal elevations variable, four to five. Egg 296 μ in length, 97 μ in width; with one polar filament. Large hooks 1940 μ in length; body length 17 - 28 mm. O. wardi, n. sp.

17 (1) Digestive tract ramifies within fixation disk. Eggs without polar filaments, but with spheroidal polar bodies 18

18 (21) Large hooks of fixation disk with five terminal elevations 19

19 (20) Terminal elevations all pointed. Egg 210 μ in length, 105 μ in width. Large hooks 1800 μ in length; body length, 12 - 15 mm.

O. batis (Cerfontaine, 1899)

20 (19) Terminal elevations not all pointed, the one next the claw being largest and very blunt. Egg 260 μ in length, 120 μ in width. Large hooks 785 μ in length; body length 8 - 9 mm.

O. prenanti St. Remy 1890

21 (18) Large hooks of fixation disk with six terminal elevations, the one farthest from the claw much larger than the remainder. Egg 225 μ in length, 120 μ in width. Large hooks 1485 μ in length; body length, 8 - 9 mm.

O. alba (Cerfontaine, 1899).

VII. The Morphology of Onchocotyle Wardi, N. Sp.

1. The General Form and Size

The external shape of the various numbers of the genus Onchocotyle has been rather aptly compared by various authors to a hammer. The body proper corresponds to the handle, the fixation disk and appendix represent the iron head and claw, the latter being well illustrated by the long appendix which is bifurcated at the free end.

The worms vary somewhat in size, specimens studied ranging from 17 to 28 mm. in length from the anterior tip to the constriction of the body to which the fixation disk is attached; from 1.5 to 3 mm. in width; and uniformly 1 mm. in thickness; the lateral direction being considered as width, and the dorso-ventral direction as thickness. The disk is oval in shape, affixed at right angles to the body proper. The disks are rather uniform in size, body variation being chiefly in the length of the body-proper. The disks are about 2 mm. wide, and 3 mm. long; the width being measured in the same plane as that of the body-proper, and the length in a plane at right angles to the width, or in a dorso-ventral relation to the body proper. The appendix varies in size from 3 to 4 mm. in length, 1 mm. in width, and 0.5 mm. in thickness. While these dimensions are average dimensions, there is very little variation from these, the greatest variation being, as noted above, in the length of the body proper.

Three distinct regions of the body are quite clearly differentiated, the body proper, the fixation disk, and the appendix. The body proper is rather uniform in width and thickness, tapering gradually at the anterior end, and more sharply at the posterior end where it is joined to the fixation disk by a narrow neck. The body is roughly ovoid in cross-section, being flat or slightly concave on the ventral side and always more or less convex on the dorsal side. The anterior sucker is situated at the anterior end of the body, slightly ventral in position. The mouth is within the sucker, at the bottom of the conical pit. The sucker is without hooks.

The genital atrium is situated on the ventral surface of the body. This is a shallow depression into which the oviduct opens, and into which the cirrus projects. Goto found no genital atrium in Onchocotyle spinacis, but in this species it is very clearly present. The cirrus and oviduct form a tiny mound in the centre of the genital atrium which is visible to the naked eye. There are no genital hooks. Just posterior to the genital atrium, on the ventral surface, and considerably off of the median line, are the two external orifices of the vaginae.

Anterior to the genital atrium, and on the dorsal surface are the external orifices of the parallel excretory canals that run thru almost the entire body. The openings are very minute and practically invisible in the toto preparations. In cross-sections, however, they are readily demonstrated.

The fixation disk at the posterior end of the body is

at once a most interesting and curious structure. On an oval disk are arranged in two arcs three pairs of suckers, the arcs extending in a dorso-ventral direction with the convexity of each arc toward the centre of the disk. Each of the six suckers contains a crescent-shaped hook, with a minute and very sharp claw on the free end. These hooks are embedded on the ventral side of the suckers. The result is that all the fixation points of the hooks extend toward the dorsal surface. The suckers are as large as the hookless anterior sucker, and the musculature indicates a surprising adhesive power.

From a mechanical viewpoint the fixation disk would be weakened by the hooks of the disks all lying in the same general direction. A disturbing force applied from the dorsal surface would tend to disengage the hooks and cause the hold to be lost. But to resist and overcome this mechanical weakness a most striking modification has developed - the appendix. The appendix is situated on the median line between the two rows of suckers, and on the dorsal side, exactly at the point of weakness. It is bifurcated at its free end, and each bifurcation has a small ellipsoidal sucker, without hooks. This pair of suckers, altho much smaller than the other three pairs, is ample to check any tendency to slip.

Unlike the Polystoma, the fixation disk does not bear hooks other than those in the suckers and on the appendix.

The hooks found in the three pairs of large suckers are crescent-shaped bodies, tapering slightly toward the buried end.

The free end of each hook has a tiny extremely sharp claw which curves inward toward the mouth of the sucker. The hooks are quite smooth, but somewhat irregular at the inner end, which is buried in the muscular wall of the sucker.

The hooks of the appendix are very tiny and readily overlooked. Each is a triradiate body, with the two shorter prongs embedded in the mesenchyma, and with the longer and extremely sharp prong free.

2. The Digestive System

The digestive system is by far the most prominent system in the body of this trematode. Due to the dark colored granular matter contained therein, the general form of the intestine may be seen in the preserved specimens without clearing, and when cleared, either with or without staining, the entire system shows up beautifully clear and distinct to very fine details.

The mouth, as previously stated, is contained within the anterior sucker, is funnel-shaped, and communicates with the pharynx by means of a very short tube (Fig. 26). The pharynx is an ellipsoidal body, and the passage thru it varies in form from elliptic to a rather irregularly shaped tube (Fig. 32). The variation is due very probably to the state of contraction at the time of death, and the circular form is the natural form. The oesophagus is rather long and communicates with the two digestive crura. There is a ventral diverticulum which underlies the phar-

ynx and extends anteriorly for a considerable distance, so that a cross-section of the pharynx quite often shows a portion of the oesophagus. There are no salivary glands.

The two crura extend thruout the length of the body proper, lying laterally near the outer edges. In the region of the constriction joining the body proper and the fixation disk, the crura reunite and the single tract continues into the fixation disk, (Fig. 25). Within the disk the tract divides again into a shorter and a longer lobe. The shorter lobe ends blindly within the disk, but the longer lobe extends into the appendix almost to its extremity. There it, too, ends blindly. The crura for a short distance posteriorly from the oesophagus are simple tubes, following the conformation of the body, which in this region is expanding to the uniform width which characterizes the body thruout its greater extent. They soon begin to branch out, however, and for the major portion of their lengths the crura are divided on the outer sides into a complicated system of lobes (Fig. 27). These lobes extend laterally, dorsally, and ventrally. At the posterior end of the body, where the crura unite into a single common duct, the lobes disappear, and the digestive tract within the fixation disk is not lobed.

The digestive tract is lined by irregularly shaped cells upon a tunica propia. The cells are very amoeboid in shape, but are generally club-shaped, connected with the wall of the intestine by narrow stalks (Figs. 29, 30). The nuclei are found in the bases of the stalks. The structure of these cells is quite

generally obscured by the great number of granular bodies within and clustered on the surfaces of the cells. These bodies range in color from a greenish brown to quite black and are innumerable. The majority seem to be on the surface of the cells.

The nature of these dark granular bodies is still in doubt. Three theories have been advanced by various investigators. First, that they are food particles. Second, that they are zymogenic and serve as agencies in facilitating digestion. The third suggestion is that they are the indigestible portion of the food materials. The possibility of the granules being food material seems very unlikely because of their uniform size, shape and appearance. Food materials would show most certainly traces of digestion, and one would expect a considerable variation in size and structure. The second theory, that the bodies are zymogenic, is more plausible, but it appears that were it correct, more of these granules would be found distributed in the lumen, and fewer clustered on the surface and within the cells. That is, if scattered thruout the food particles the action would be facilitated, whereas the action must necessarily be localized when they are grouped in clusters. The third explanation, that they are the indigestible portion of the food is also very plausible, but again one would not expect to find such a uniform size, color, and structure.

When the various positions of these granules are considered, within the cells, clustered on the surfaces of the cells, and in clusters thruout the lumen, the possibility of their being

excretory products is rendered very probable. I think it probable that the club-shaped portions of the cells are gradually filled with these excretory bodies and that they then become detached from the narrow stalks and are expelled from the lumen by way of the mouth. Such bodies are quite numerous in the lumen, identical in appearance with similar bodies attached by stalks to the intestinal wall. Some of these bodies are nucleated. The characteristic contractions and relaxations of the whole body would tend to expel the fluids of the lumen and with them the excretory bodies.

3. The Excretory System

The excretory system is very difficult to demonstrate, except in its general features. In toto mounts no trace of an excretory system can be discovered, and in the cross-sections only the main ducts may be distinguished with certainty. There are two main ducts on each side of the body, a greater and a lesser. The larger of these ducts open to the exterior on the dorsal side anterior to the genital atrium. These orifices are so tiny that they cannot be seen externally, due most likely to the contraction of the body when it is fixed. They can be demonstrated readily, however, in sections (Fig. 35). The ducts have walls of a structureless tissue, and are embedded in the mesenchyma. They are ventral to the digestive crura thruout most of the length of the body, rising gradually in the vicinity of the genital atrium to the dorsal surface where the larger open to the exterior (Fig. 35).

There is a considerable variation in the diameter of these ducts thruout their course, but in the oesophageal region the two communicating with the exterior are considerably expanded into what may be termed bladders, narrowing down, however, before reaching the orifices. Occasionally traces of smaller ducts, and doubtless tributary to the main ducts may be seen in the mesenchyma. Both pairs of ducts penetrate a considerable distance into the fixation disk, following closely the digestive tract. No traces of the flame cells were found.

4. The Reproductive System

Next to the digestive tract in prominence is the reproductive system, occupying the space between the digestive crura thruout the length of the body, and staining in part so deeply as to be very conspicuous.

The male reproductive system is relatively simple. The many-lobed testes occupy the posterior half of the space between the crura, and show little regularity. The lobes are thin walled, of structureless material, and vary up to seven in number, and considerably in size, as is shown by cross-section. The cavities are filled with the sperm-cells and the accompanying nurse cells in various stages of development. From the testes extend anteriorly two ducts, the vas efferens, which unite on the left ventral side of the body and continue forward as the vas deferens. The vas deferens enlarges considerably in the anterior half of the

body, and is coiled and doubled back on itself in a peculiar fashion. No two specimens show an identical in this coiling. This enlarged, coiled portion is usually filled with sperm masses. It communicates with the cirrus by a large straight duct which lies just beneath the uterus, (Fig. 28). The cirrus is quite large and projects into the uterus and the genital atrium as described later on. The duct of the cirrus appears to be ciliated, (Figs. 11, 12, Plate II), the vas deferens certainly is not.

The female reproductive system occupies the anterior half of the body and is very complex (Fig. 28). The ovary is found on the right side of the body, and approximately midway between the extremes of the body proper. It is an irregularly shaped body which doubles upon itself and communicates with the ootype by means of a short oviduct. The ovary when sectioned is seen to consist of germinal cells in various stages of maturity. The earliest stage (Fig. 16) is that of a compact mass of cells, with or without nuclei, and of irregular shapes. Farther down the ovary, i.e., nearer the oviduct, other stages (Figs. 17, 18, 19) may be discerned, in which the mature egg-cells are embedded in a structureless matrix which breaks up and liberates the matured cells. The ovary communicates with the ootype by means of a short oviduct. Cross-sections of the oviduct appear to be ciliated, but closer examination shows that the lining is of amoeboidal cells, having large distinct nuclei, each with a definite nucleolus.

The receptaculum seminale is a large thin-walled oval sac connected to the oviduct by a short tube which has two distinct enlargements, which in cross-section show cross-filaments, that appear to be muscular fibers, but I have not been able to demonstrate them as such. If they are muscles, they would serve as valves, opening and closing the duct which communicates with the oviduct and thereby controlling fertilization. The receptaculum seminale lies opposite the ovary on the left side of the body. In all specimens examined the receptaculum seminale was almost completely filled with sperm masses which stain intensely.

Communicating with the ootype are also the genito-intestinal canal, and the yolk duct which is also the medium of communication with the vaginae. The genito-intestinal canal is a short duct with rather thin walls that leads directly into the right digestive crus. In cross-section both nuclei and nucleoli can be made out readily, altho Goto describes it as wholly destitute of nuclei. He mentions a lining with fine cillia which I have not been able to demonstrate. The opening into the intestine is very minute (Figs. 20, 21, 22, 23), and shows no evidence of a valve. The ootype is a spindle-shaped body, surrounded by shell glands (Fig. 33). In cross-section it presents a stellate appearance due to the thickenings of the lining membrane. In each of the thickenings is a nucleus with a distinct nucleolus. The wall of the ootype is a thin darkly staining structure without nuclei (Fig. 15).

The uterus is a small straight duct leading from the

ootype to the genital atrium. It is a relatively thick-walled duct, showing nuclei and nucleoli, but as is true of all tissues in the parasite, no cell outlines (Fig. 10). It is ciliated thruout its course. Shortly before the genital atrium, the uterus enlarges dorsally. It is at this place the cirrus enters the uterus. In cross-sections this may be seen very clearly, the uterus being a large, thin-walled tube with the thick-walled cirrus occupying the ventral side (Fig. 11). Farther on the cirrus is seen lying wholly within the uterus (Fig. 12). This enlargement leads into the genital atrium, into which the cirrus projects as a small conical body (Figs. 4 and 5).

The vitellaria are two multilobed bodies extending thruout the major portion of the body. By means of cross-sections the vitellaria are seen to extend from the anterior tapering portion of the body back posteriorly to almost the region in which the digestive crura reunite. The vitellaria surround the crura, but the lobes are more numerous on the dorsal side. The lobes are filled with the yolk-granules, tiny spherical bodies of a greenish yellow color. The lobes have very thin walls, which are often very difficult to demonstrate. The two yolk ducts leading from the lateral vitellaria unite in the median line, and a single yolk duct leads to the ootype. The paired yolk ducts are usually enlarged into yolk reservoirs which store the yolk material until needed.

The vaginae are paired ducts which have their external orifices on the ventral surface just anterior to the genital atrium

and situated laterally to it. The orifices are very minute and are made out with difficulty in the unstained and uncleared whole worms. They are, however, readily found in the cross-sections (Fig. 14). From these orifices two small ducts lead posteriorly, enlarging immediately to considerable size, and then tapering as they extend posteriorly. Quite often one or two constrictions will be present in the vaginae. The vaginae are ciliated (Fig. 13) and lead into the paired yolk ducts of the vitellaria, which communicate by means of the single yolk duct with the ootype and receptaculum seminale.

The mature eggs are found in the uterus and are never numerous. From one to two are found in a single specimen. The eggs are probably passed out as soon as fully mature. The eggs are ovoid, tapering at each end, with polar filaments which vary considerably in length on the same and different individuals. The eggs average about 296 microns in length, exclusive of the variable polar filaments.

The process of reproduction is probably as follows: The sperms are formed in great numbers and are stored in the multi-lobed seminal vesicle. In coitus the sperm masses are introduced into the vaginae by means of the cirrus. Aided by the ciliated inner surfaces of the vaginae, the sperm masses pass posteriorly into the lateral yolk ducts, and from them into the common median yolk duct. This communicates with the vesicle formed by the common junction of the yolk duct, oviduct, ootype, and genito-intestinal canal. From this vesicle the sperm masses

pass into the oviduct and are stored in the receptaculum seminale. The egg-cells as they are formed in the ovary pass down the oviduct, are fertilized in the oviduct, and then pass into the ootype. There the yolk material is added, and the enclosing shell added. The completed egg passes into the uterus, and by the ciliary action is carried anteriorly to the genital atrium where it is liberated.

The part played by the genito-intestinal canal is as yet in doubt. There is no agreement among investigators as to its function. The suggestion that it is a passage way thru which surplus yolk material is excreted has been offered, but not proven, and does not appear very plausible.

5. The Nervous System

Scarcely anything of the nervous system can be demonstrated. Above the oesophagus are two conspicuous ganglia, from which nerves may be traced a short distance anteriorly and very little farther posterior. The two lateral nerve trunks may be traced for a greater distance. Apparently there is little or no difference between the nervous system of this species and O. borealis as far as can be determined.

VIII. Conclusions

1. The genus Onchocotyle belongs to the family *Polystomidae* van Beneden, of the order *Polyopisthocotylea* Odhner.

2. A new species, *O. wardi*, is described, resembling most nearly *O. borealis*.

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X. Explanation of Plates

All drawings were made with the camera lucida, and then reduced approximately one-half.

Plate I

Figure 1. Ventral view of complete specimen. x 6

Figure 2. Left lateral view of complete specimen. x 6

Figure 3. Anterior sucker. x 9

Figure 4. Cirrus. x 9

Figure 5. Anterior portion of the body (ventral), showing sucker, genital atrium, and orifices of vaginae. x 9

Figure 6. Fixation disk and appendix. x 9

Figure 7. Fixation disk and appendix. x 9

Figure 8. Section thru posterior sucker. x 52
h. section of hook

Figure 9. Two hooks from posterior suckers, isolated with lactic acid.

Plate II.

Figure 10. Uterus in cross-section. x 609

Figure 11. Uterus, with cirrus entering it. x 304

Figure 12. Uterus, with cirrus completely within it. x 304

Figure 13. Vagina in cross-section. x 477

Figure 14. Opening of vagina to exterior. x 304

Figure 15. Ootype in cross-section x 207

Figures 16, 17, 18, 19. Sections through the ovary, showing stages in maturing of the eggs. x 477

Figures 20, 21, 22, 23. Serial sections through genito-intestinal canal at the point of emptying into the right digestive crus. x 304

Figure 24. Oviduct in cross-section. x 609

Plate III.

Figure 25. The digestive crura. x 6

Figure 26. Anterior sucker, mouth, pharynx, oesophagus and anterior ganglia. x 118

Figure 27. Longitudinal section thru digestive crus. x 37

Figure 28. Schematic view of reproductive systems.

d.cr.	right digestive crus
g.i.	genito-intestinal canal
oot.	ootype
ov.	oviduct
r.s.	receptaculum seminale
tes.	testes
ut.	uterus
vag.	vagina
v.d.	vas deferens
vit.	vitellaria

Figure 29. Lining of digestive crus. x 612

Figure 30. Mature eggs from uterus. x 47

Figure 31. Cells of digestive crus. x 612

Figure 32. Pharynx in cross-section. x 128

Figure 33. Cross-section of the body in the region of the ootype. x 41

oot. ootype

s.g. shell gland

y.d. yolk duct

Figure 34. Cross-section of the body in the region of the receptaculum seminale. x 41

d.cr. digestive crus

ov. ovary

r.s. receptaculum seminale

vit. vitellaria

Figure 35. Excretory duct in cross-section, and external orifice. x 238.

Plate I

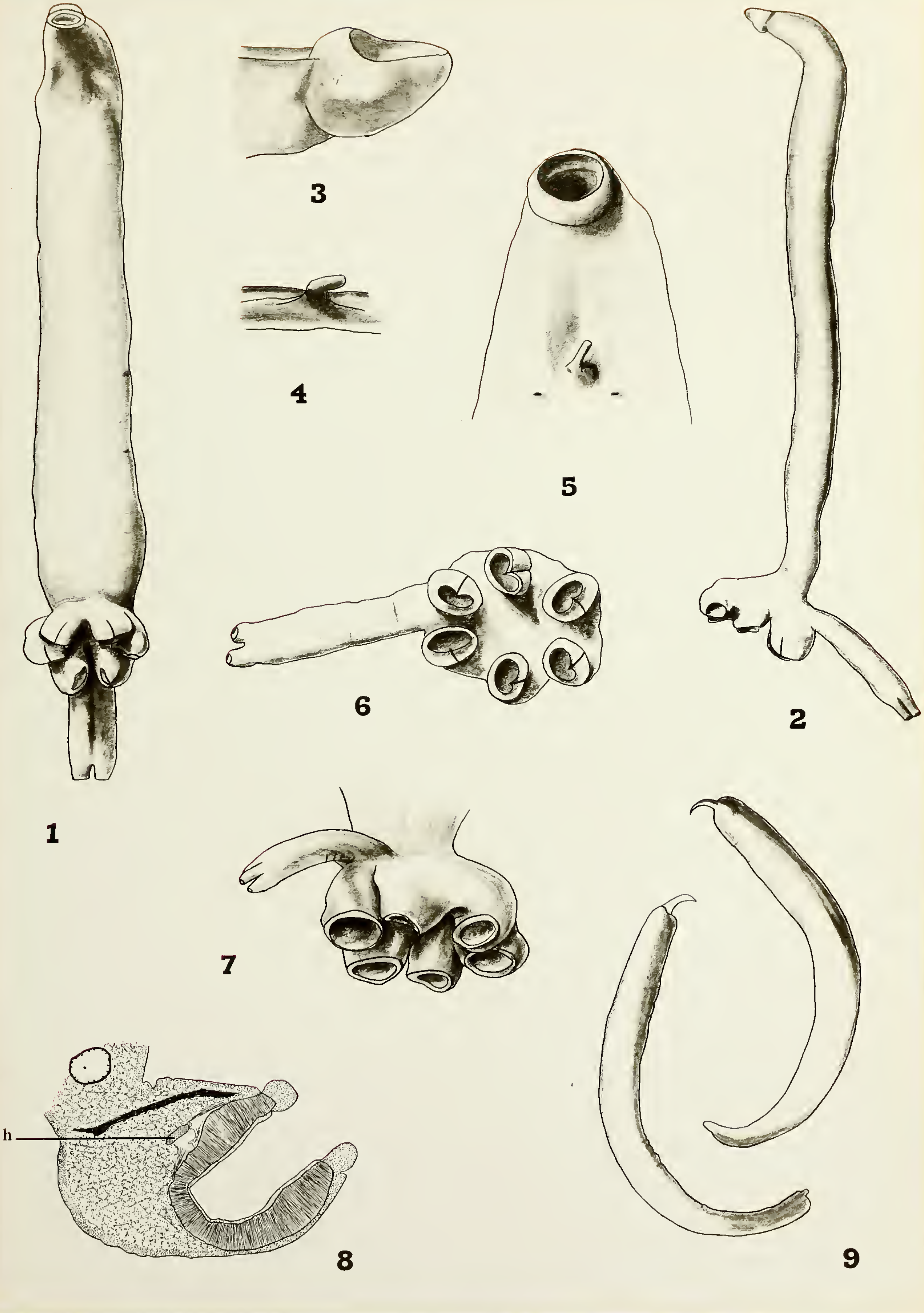

Plate II

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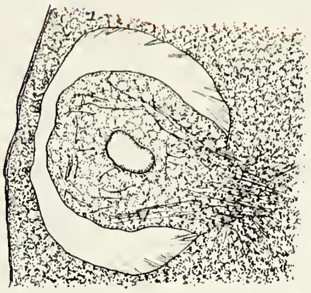

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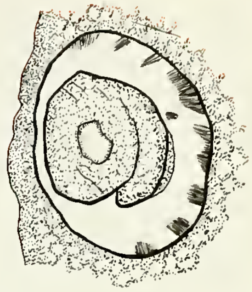

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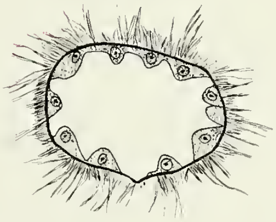

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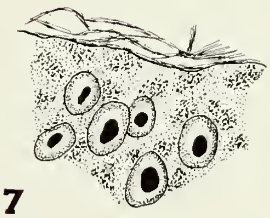

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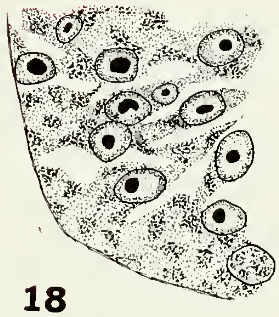

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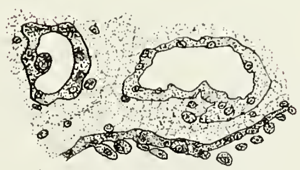

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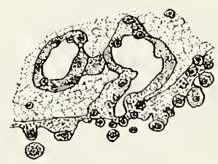

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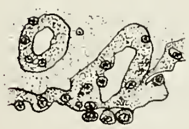

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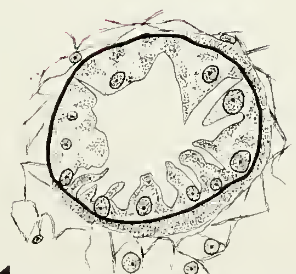

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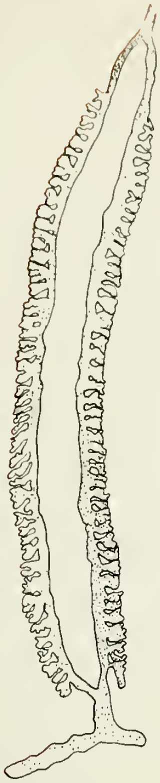
Plate III.



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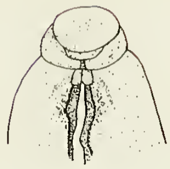

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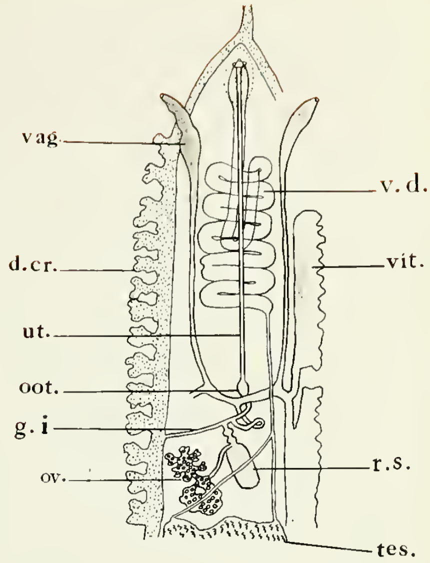

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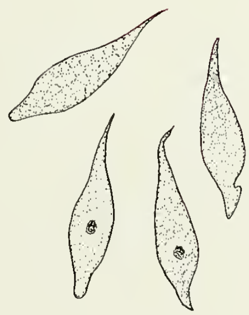

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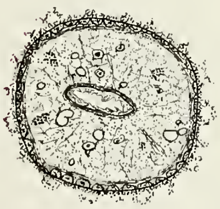

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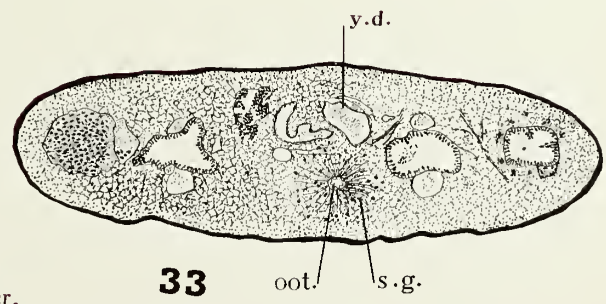

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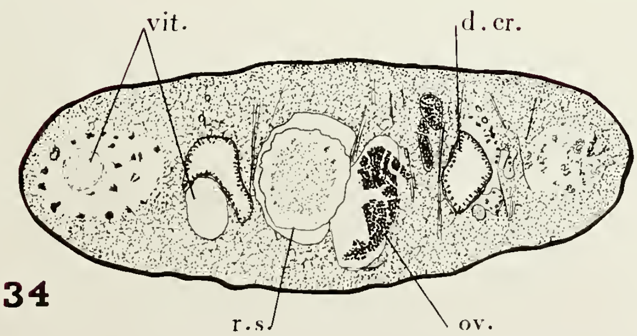

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